Molecular phylogeny of anoplocephalid tapeworms (Cestoda: Anoplocephalidae) infecting humans and non-human primates

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SUMMARY

Anoplocephalid tapeworms of the genus *Bertiella* Stiles and Hassall, 1902 and *Anoplocephala* Blanchard, 1848, found in the Asian, African and American non-human primates are presumed to sporadic ape-to-man transmissions. Variable nuclear (5.8S-ITS2; 28S rRNA) and mitochondrial genes (*cox*1; *nad*1) of isolates of anoplocephalids originating from different primates (*Callicebus oenanthe, Gorilla beringei, Gorilla gorilla, Pan troglodytes* and *Pongo abelii*) and humans from various regions (South America, Africa, South-East Asia) were sequenced. In most analyses, *Bertiella* formed a monophyletic group within the subfamily Anoplocephalinae, however, the 28S rRNA sequence-based analysis indicated paraphyletic relationship between *Bertiella* from primates and Australian marsupials and rodents, which should thus be regarded as different taxa. Moreover, isolate determined as *Anoplocephala* cf. *gorillae* from mountain gorilla clustered within the *Bertiella* clade from primates. This either indicates that *A. gorillae* deserves to be included into the genus *Bertiella*, or, that an unknown *Bertiella* species infects also mountain gorillas. The analyses allowed the genetic differentiation of the isolates, albeit with no obvious geographical or host-related patterns. The unexpected genetic diversity of the isolates studied suggests the existence of several *Bertiella* species in primates and human and calls for revision of the whole group, based both on molecular and morphological data.

Key words: *Bertiella*, *Anoplocephala*, phylogeny, primates, zoonotic potential.

INTRODUCTION

The adult stages of tapeworms of the cyclophyllidean family Anoplocephalidae Cholodkovsky, 1902, are intestinal parasites of mammals, birds and reptiles (Beveridge, 1994). In primates, several species of the subfamily Anoplocephalinae mostly from the genera *Anoplocephala, Bertiella* and *Moniezia* Blanchard, 1891, represent typical components of parasite communities. However, occasional infections by representatives of the subfamilies Linstowiinae (*Mathevotaenia* Akhumian, 1946) and Inermicapsiferinae (*Thysanotaenia* Beddard, 1911) have also been reported (Beveridge, 1994). Although the genus *Anoplocephala* mostly includes species from hyraxes, odd-toed ungulates and elephants, *Anoplocephala gorillae* Nybelin, 1927, was described from eastern gorillas (*Gorilla beringei*) and is considered to be a common parasite in the Rwandan and Ugandan mountain gorilla populations (Sleeman *et al*. 2000; Rothman *et al*. 2008). In contrast, almost no cestodes have been found in western gorillas (*Gorilla gorilla*) of Gabon

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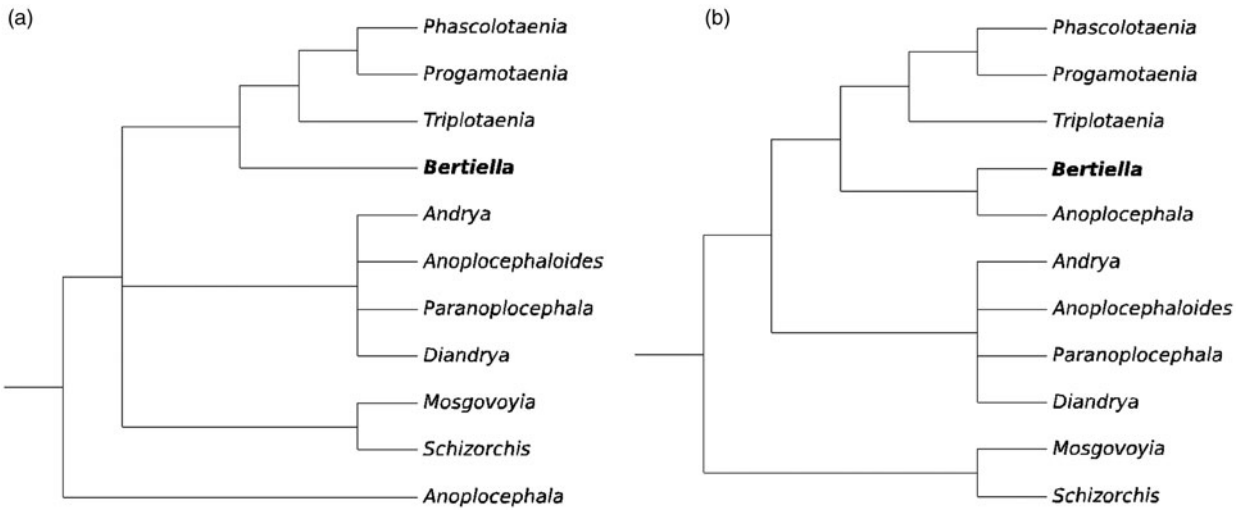

Fig. 1. The phylogenetic trees of anoplocephaline cestodes: (A) position of *Bertiella* based on morphological-characters (Beveridge, 1994); (B) position of *Bertiella* based on analysis of the 28S rRNA gene (Hardman *et al*. 2012).

(Landsoud-Soukate *et al*. 1995) and in *G. gorilla* from various localities in the Congo basin (author's unpublished data); only Lilly *et al*. (2002) reported anoplocephalid eggs in two fecal samples of *G. gorilla* in the southern Dzanga-Ndoki National Park, Central African Republic, yet without specific identification. Other anoplocephalids, such as *Moniezia rugosa* Lühe, 1895, were encountered rather exceptionally in New World primates from the families Atelidae and Cebidae (Dunn, 1963; Fiennes, 1967), with no recent records being available.

More common tapeworms in primates are members of the genus *Bertiella*, although other members of this genus parasitize a broad spectrum of mammalian hosts including marsupials, flying lemurs and rodents in Africa, Asia, Australia and South America (Denegri and Perez-Serrano, 1997). The type species, *Bertiella studeri*, was described from a common chimpanzee *Pan troglodytes* by Blanchard (1891). Thereafter, more than 40 species have been assigned to the genus (Schmidt, 1986; Caira *et al*. 2012), ten of them being known to infect primates (Schmidt, 1986; Denegri and Perez-Serrano, 1997; Galán-Puchades *et al*. 2000). Infections by three of the typical primate tapeworms, *B. studeri* (Blanchard, 1891), *Bertiella mucronata* (Meyner, 1895) and *Bertiella satyri* (Blanchard, 1891), are sporadically reported also from humans (Chandler, 1925; Cameron, 1929; Denegri and Perez-Serrano, 1997; Bhagwant, 2004; Foitová *et al*. 2011). Human infections with *B. studeri* were reported mostly from India, the Russian Far-East and Africa (Bhagwant, 2004; El-Dib *et al*. 2004), or from non-residents that visited these regions (Cameron, 1929; Galán-Puchades *et al*. 2000), while *B. mucronata* and *B. satyri* were confined to men and primates living in South America and South-East Asia, respectively (Gómez-Puerta *et al*. 2009; Foitová *et al*. 2011). Diagnostics of the anoplocephaline infections are based mostly on the identification of gravid proglottids or eggs that possess a typical pyriform apparatus (Schmidt, 1986; Denegri and Perez-Serrano, 1997); yet identifying these parasites to the species level in clinical material from primates remains rather difficult.

In the last decade, molecular approaches helped to resolve the phylogenetic relationships of several genera of the Anoplocephalinae, including *Bertiella* (see e.g. Haukisalmi *et al*. 2010; Hardman *et al*. 2012; Taleb-Hossenkhan and Bhagwant, 2012). Not surprisingly, molecular analyses showed that previous morphology-based classification may not reflect the actual relationships within the subfamily. In morphology-based phylogeny by Beveridge (1994), *Bertiella* and *Triplotaenia* Boas, 1902, were sister genera, whereas the genus *Anoplocephala* was basal taxon of the subfamily (Fig. 1A). Molecular-based phylogeny by Hardman *et al*. (2012), however, showed a sister relationship between *Bertiella* and *Anoplocephala*, with the genera *Schizorchis* Hansen, 1948, and *Mosgovoyia* Spasskii, 1951, emerging as basal taxa (Fig. 1B). The genus *Bertiella* deserves special attention, as previous studies did not include any isolates from primates (Hardman *et al*. 2012), or the used molecular marker was not sufficient for the establishment of intra-generic relationships within the cestodes (Taleb-Hossenkhan and Bhagwant, 2012).

The present study provides the first molecular insight into the diversity of *Bertiella* and *Anoplocephala* from humans and non-human primates from Africa, Asia, Europe and South America, and describes their host specificity and phylogenetic relationships.

Table 1. The locality, source of DNA and GenBank accession numbers of particular genes of cestodes from non-human primates and humans. The extended dataset includes an isolate from horse

Cestode isolate	Host species/ locality	Habitat	Source of DNA	*cox*1	*nad*1	5.8S-ITS2	28S
Bertiella sp.	*H.s.* BR	c/s	Proglottid	–	JQ771110	JQ771093	
Bertiella studeri[a]	*H.s.* ES	c/s	Proglottid	JQ771102	JQ771111	JQ771094	
Bertiella satyri[b]	*P.a.* ID	c/s	Proglottid	JQ771103	JQ771112	JQ771195	
Bertiella mucronata[c]	*C.o.* PE	c/s	Proglottid	JQ771104	JQ771113	JQ771096	
Bertiella sp.	*P.t.* UG	w	Proglottid	JQ771105	JQ771114	JQ771097	KJ888951
Bertiella sp.	*P.t.* KE	c/s	Proglottid	JQ771106	JQ771115	JQ771098	
Bertiella sp.	*P.t.* GW	w	Eggs	–	–	–	
Bertiella sp.	*G.g.* CF	w	Proglottid	–	JQ771116	JQ771099	KJ888952
Anoplocephala cf. *gorillae*	*G.b.* RW	w	Eggs	–	JQ771117	JQ771100	
Anoplocephala perfoliata	*E.c.* CZ	c/s	Eggs	JQ771109	JQ771118	–	

H.s., *Homo sapiens*; P.a., *Pongo abelii*; C.o., *Callicebus oenanthe*; P.t., *Pan troglodytes*; G.g., *Gorilla gorilla*; G.b., *Gorilla beringei*; E.c., *Equus cabalus*; ES, Spain, BR, Brazil; ID, Indonesia; PE, Peru; UG, Uganda; KE, Kenya; GW, Guinea-Bissau; CF, Central African Republic; RW, Rwanda; CZ, Czech Republic; c/s, captivity/sanctuary; w, wild.
[a] Galán-Puchades *et al*. (2000).
[b] Foitová *et al*. (2011).
[c] Gómez-Puerta *et al*. (2009).

MATERIALS AND METHODS

Sample collection and preparation

The proglottids of anoplocephalid tapeworms were acquired from the following hosts and localities: (i) wild eastern chimpanzee *P. troglodytes schweinfurthii* from Uganda (Kalinzu Forest Reserve), (ii) semi-captive chimpanzees from Kenya (Sweetwaters Chimpanzee Sanctuary), (iii) wild western lowland gorilla *Gorilla gorilla gorilla* from the Central African Republic (Dzanga-Ndoki National Park), (iv) semi-captive Sumatran orangutan *Pongo abelii* from an Indonesian sanctuary (Gunung Leuser National Park), (v) captive Titi monkey *Callicebus oenanthe* from Peru (Moyobamba) and, (vi) humans from Brazil and Spain. The Spanish isolate from human and isolates from *C. oenanthe* and *P. abelii* were previously identified as *B. studeri*, *B. mucronata* and *B. satyri*, respectively, based on morphological characteristics of whole tapeworms or proglottids (Galán-Puchades *et al*. 2000; Gómez-Puerta *et al*. 2009; Foitová *et al*. 2011). Furthermore, fecal samples containing cestode eggs with a characteristic pyriform apparatus (Beveridge, 1994) were collected from wild western chimpanzee *P. troglodytes verus* from Guinea-Bissau (Cantanhez National Park; Sá *et al*. 2013), and also from wild mountain gorilla *Gorilla beringei beringei* from Rwanda (Volcanoes National Park). The morphology of the eggs of isolate from mountain gorilla corresponds to eggs of *A. gorillae* previously identified by Sleeman *et al*. (2000). In addition, eggs of *Anoplocephala perfoliata* isolated from domestic horse *Equus cabalus* (Brno, Czech Republic) were included into the study (summary of all isolates in Table 1).

Proglottids and fecal samples (~2 g) were preserved in 96% ethanol. The proglottids collected from feces were washed 5 times in phosphate buffered saline (PBS), disrupted with micro-pestles and washed 5 times in PBS followed by centrifugation at ~16 000 ***g*** for 5 min before DNA was extracted from the pellet. Feces were processed by flotation technique with modified Sheather's solution (Sheather, 1923). The eggs from surface of flotation solution were collected with a wire loop and transferred to a 10 mL vial tube containing 8 mL of PBS. Then, the vial was centrifuged at ~320 ***g*** for 2 min and the supernatant was carefully removed. The sediment was resuspended in 1 mL of PBS and 40 μL were transferred onto a microscopic slide with a dimple. Individual eggs were isolated with a thin glass micropipette, normally used for embryo transfers that had a short silicone hose with a 2 mm inner diameter and node on one end. The eggs (minimum 30) were transferred into 0·5 mL of PBS in a new 2 mL centrifuge tube. Prior to DNA extraction, the egg shells were disrupted with glass beads (0·5 and 1 mm) in a BeadBeater (Biospec, USA) by shaking at 2400 oscillations min^{-1} for 10 min.

DNA extraction

Before DNA extraction, 800 μL of NET buffer (4 M NaCl; 0·5 M Ethylenediaminetetraacetic acid; 1 M Tris, pH 8·0), 240 μL of N-lauroylsarcosine sodium salt solution (Sigma Aldrich, Germany) and 30 μL of proteinase K in concentration 100 μg mL^{-1} (Chemos, Czech Republic) were added to the sample. The homogenate was incubated in a dry bath at 56 °C for 15 h, and the lysate was subsequently extracted by

phenol–chloroform. An equal volume of phenol (Sigma Aldrich, Germany) was added; gently vortexed for 10 min, spun for 10 min, and the procedure was repeated with equal volumes of phenol and subsequently chloroform (Sigma Aldrich, Germany). Following the extraction, DNA was precipitated with ethanol and sodium acetate, air-dried and resuspended in water.

Polymerase chain reaction (PCR) amplification and sequencing

For the purpose of identification of isolates as belonging to the genus *Bertiella*, we designed the primers amplifying 600 bp of 18S rRNA based on the published sequence of *B. studeri* obtained from a morphologically identified adult tapeworm from the crab-eating macaque *Macaca fascicularis* by Taleb-Hossenkhan and Bhagwant (2012). Using forward primer BF (5′-GGACACTATGAGGA TTGACAGA-3′) and reverse primer BR (5′-CC TTTCGGGGCACCAAGATGG-3′), we amplified the fragment of 18S rRNA from our samples under following PCR conditions: an initial denaturation at 96 °C for 3 min and then 30 cycles of 1 min at 94 °C, 1 min at 58 °C and 1 min at 72 °C, followed by 10 min at 72 °C. These sequences obtained were later compared with the published sequence of *B. studeri*.

For further phylogenetic and taxonomic comparison, the cytochrome c oxidase subunit I (*cox*1) and partial nicotinamide adenine dinucleotide dehydrogenase dehydrogenase subunit 1 (*nad*1) of mitochondrial (mt) DNA, 5.8S-ITS2 and partial 28S rRNA of nuclear (n) DNA were sequenced, as published comparative sequences of these markers are available in larger abundance and broader taxonomic spectrum. The *cox*1 gene fragment was amplified using primers COX-F (5′-GATGTTTTCTTT ACATTTATCTGGTG-3′) and COX-R (5′-GCCACCACAAATCAAGTATC-3′) following the protocol of Haukisalmi *et al.* (2004). The *nad*1 gene fragment was amplified by primers Cyclo-Nad1F (5′-GGNTATTSTCARTNTCGT AAGGG-3′) and Cyclo-trnNR (5′-TTCYTGAAG TTAACAGCATCA-3′) under conditions described elsewhere (Littlewood *et al.* 2008). For 5.8S-ITS2 region primers Proteo1 (5′-CGGTGGATCACTC GGCTC-3′) and Proteo2 (5′-TCCTCCGCTTATT GATATGC-3′) designed by Škeříková *et al.* (2004) were used for 40 cycles (1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C) amplification. Additionally, the D1–D3 region of 28S rRNA gene was amplified using three alternative pairs of primers modified by Hardman *et al.* (2012): (i) LSU5 (5′-TAGGTCG ACCCGCTGAAYTTYAGCA-3′) and 1200R (5′-GCATAGTTCACCATCTTTCGG-3′) (*c.* 1400 bp); (ii) XZ-1 (5′-ACCCGCTGAATTTAAGCA TAT-3′) and 1500R (5′-GCTATCCTGAGGG AAACTTCG-3′) (*c.* 1660 bp) and (iii) U178 (5′-G CACCCGCTGAAYTTAAG-3′) and L1642 (5′-CCAGCGCCATCCATTTTCA-3′) (*c.* 1500 bp). As used by Hardman *et al.* (2012), the PCR conditions were following those of Lockyer *et al.* (2003), Waeschenbach *et al.* (2007) and Littlewood *et al.* (2008), respectively . Upon resolution in ethidium bromide-stained agarose gels, the amplicons were gel-purified using the QuickClean Gel Extraction Kit (GenScript, USA) and sequenced at Macrogen Inc. (Seoul, Korea). The assembled nucleotide sequences have been deposited in GenBank under the accession numbers JQ771093–JQ771118 and KJ888951–KJ888952 (Table 1).

Phylogenetic analyses

For phylogenetic analyses, we aligned seven 5.8.S-ITS2, 53 *cox*1, 17 *nad*1 and 24 28S rRNA GenBank sequences of anoplocephaline species (accession numbers of sequences are shown in the trees) with newly obtained tapeworm sequences from humans, non-human primates and horse, respectively. Based on availability in GenBank, sequences of *Echinococcus* spp. or *Hymenolepis* spp. were included as outgroup. Sequences of the respective genes were aligned in BioEdit (Hall, 1999) using the Clustal W programme (Larkin *et al.* 2007), with a default scheme for introducing gaps into the alignment, and were further checked for inconsistencies and manually edited, when needed. Phylogenetic relationships among the *Bertiella* and *Anoplocephala* isolates and selected anoplocephaline species were estimated using Bayesian inference (BI) in the MrBayes 3.2 software (Ronquist and Huelsenbeck, 2003) under substitution models suggested by MrModeltest 2.3 (Nylander, 2004). Two independent runs were executed in each BI, lasting 1 million generations and sampled every 100 generations. The burn-in portion of the sampled trees was set to the default 25%. The mitochondrial and nuclear markers were analysed separately because of different included taxa available for each of them.

Mitochondrial *cox*1 (561 bp) and *nad*1 (747 bp) were analysed under the HKY+I+G and GTR+I +G substitution models, respectively. The large *cox*1 dataset containing multiple conspecific sequences of various anoplocephalid tapeworm genera was further used to assess the taxonomic significance of the genetic variability within our isolates based on the variation in percentage genetic distances within and between relevant clades representing distinct species. In addition to the 747 bp *nad*1 dataset, a portion thereof trimmed to 366 bp was alternatively analysed in order to include a shorter sequence of an isolate from *G. beringei* from Rwanda, which, despite repeated attempts of amplification and sequencing, could not be obtained in full length.

Due to short length of 5.8S rRNA, the nuclear 5.8S-ITS2 (896 bp after introduction of gaps) was

analysed as an unpartitioned dataset under HKY+I +G substitution model.

Nuclear 28S rRNA sequences of *Bertiella* from primates were aligned together with the published data by Hardman *et al*. (2012) in order to compare our data with published information on *Bertiella* from Australian marsupials and rodents. This alignment (1386 bp after introduction of gaps) was manually edited and ambiguously aligned positions were excluded prior to analysis, yielding a 1161 bp long alignment, which was analysed under GTR+I+G model using BI.

RESULTS

Low amount of available DNA was a limiting factor of our studies and in the case of *P. troglodytes verus* from Guinea-Bissau, we failed, despite multiple attempts, to obtain sufficient amount of DNA. In total, we managed to obtain eight sequences of 18S rRNA, six sequences of *cox*1, eight of *nad*1, eight of partial 5.8S-ITS2 rRNA and finally, two of 28S rRNA genes. The length of 18S amplicons varied from 680 to 710 bp, those of *cox*1 amplicons from 585 to 680 bp and *nad*1 from 730 to 900 bp, the size of partial 5.8S-ITS2 rRNA sequences obtained ranged from 620 to 820 bp and the size of 28S rRNA amplicons was 1460–1500 bp.

In 18S rDNA sequences, the query coverage of all our isolates with respect to the published sequence of *B. studeri* (GU323706) was 100% and the sequence identity reached as much as 97% for isolate from *G. gorilla*, 98% for isolates from Ugandan *P. troglodytes, G. beringei, C. oenanthe* and Brazilian isolate from human, and 99% for isolates from Kenyan *P. troglodytes* and *P. abelii*. An identity of 100% was revealed for Spanish isolate from human, which was previously determined as *B. studeri* by Galán-Puchades *et al*. (2000).

The topology of the *cox*1 (Fig. 2), *nad*1 (Fig. 3A) and 5.8S-ITS2 (Fig. 4) trees showed that all isolates morphologically determined as *Bertiella* clustered together in a monophyletic group. This *Bertiella* cluster included also the isolate from *G. beringei* from Rwanda, morphologically determined as *Anoplocephala* cf. *gorillae*, from which only a partial fragment of *nad*1 was acquired (Fig. 3B). The internal topology of the *Bertiella* clade varied according to the respective markers and phylogenetic methods used, but in most analyses, no correlation with host or geography was observed. This means that cestodes from the same (humans) or congeneric hosts (two gorillas or three chimpanzees) were not closely related. In contrast, isolates from unrelated hosts and different locations were closely related, such as those of *B. mucronata* from *C. oenanthe* from Peru and *Bertiella* sp. from *P. troglodytes* from Uganda, or those from a Brazilian human and *P. troglodytes* from Kenya (Figs 2–4). Variability among *Bertiella* haplotypes expressed as pairwise genetic distance of the *cox*1 gene (Fig. 2) was 0·2–12·0% (Table 2).

The analysis of partial 28S rRNA showed that the two *Bertiella* isolates from *P. troglodytes* from Uganda and *G. gorilla* do not cluster with other *Bertiella* isolates from marsupials and rodents, but form a separate clade, turning the genus into a paraphyletic assembly. As a consequence, the genus *Anoplocephala* represents a sister group of *Bertiella* spp. from marsupials and rodents (Fig. 5).

DISCUSSION

Despite the fact that anoplocephalid cestodes are known as parasites of humans, primates and other warm-blooded vertebrates for a long time, their taxonomy has far been unsatisfactorily addressed, especially in case of species parasitizing hosts other than rodents. Resolving the taxonomy of species from primates including man is complicated by the fact that the gravid proglottids and eggs obtained from the feces are mostly the only stages diagnosed, whereas the scolexes and larger parts of the strobila, including mature proglottids with taxonomically important structures, are not available (Beveridge, 1994; Sun *et al*. 2006).

In present study, involving anoplocephalid cestodes from several primate hosts from different continents, the phylogenetic analyses based on nuclear (5.8S-ITS2) and mitochondrial (*cox*1, *nad*1) genes showed that all isolates acquired from humans and primates form a monophyletic group. Such a situation is in agreement with an 18S rRNA-based study of human *Bertiella* isolates and those from the crab-eating macaque *M. fascicularis* (Furtado *et al*. 2012; Taleb-Hossenkhan and Bhagwant, 2012). However, clustering within this monophyletic group was inconsistent, probably as a consequence of a low number of, and/or high variability among, the *Bertiella* isolates. Thus, using the data of Haukisalmi *et al*. (2004, 2007), we compared the genetic distances within and among the genera *Paranoplocephala* Lühe, 1910, *Microcephaloides* Haukisalmi, Hardman, Hardman, Rausch and Henttonen, 2008, *Anoplocephaloides* Baer, 1923 and *Bertiella*. The results revealed higher heterogeneity of *Bertiella* compared with that of the other genera, indicating the presence of several species within our dataset (Table 2). Although our collection of isolates from man and primates included also morphologically determined specimens, the isolates studied herein could not be referred to a particular *Bertiella* species. They are apparently congeneric; however, the lack of clear correlation between hosts or geography makes the identification to the species level impossible.

In morphology-based phylogenetic analyses of the Anoplocephalinae (Beveridge, 1994), the genus *Anoplocephala* appeared as a basal clade for all the

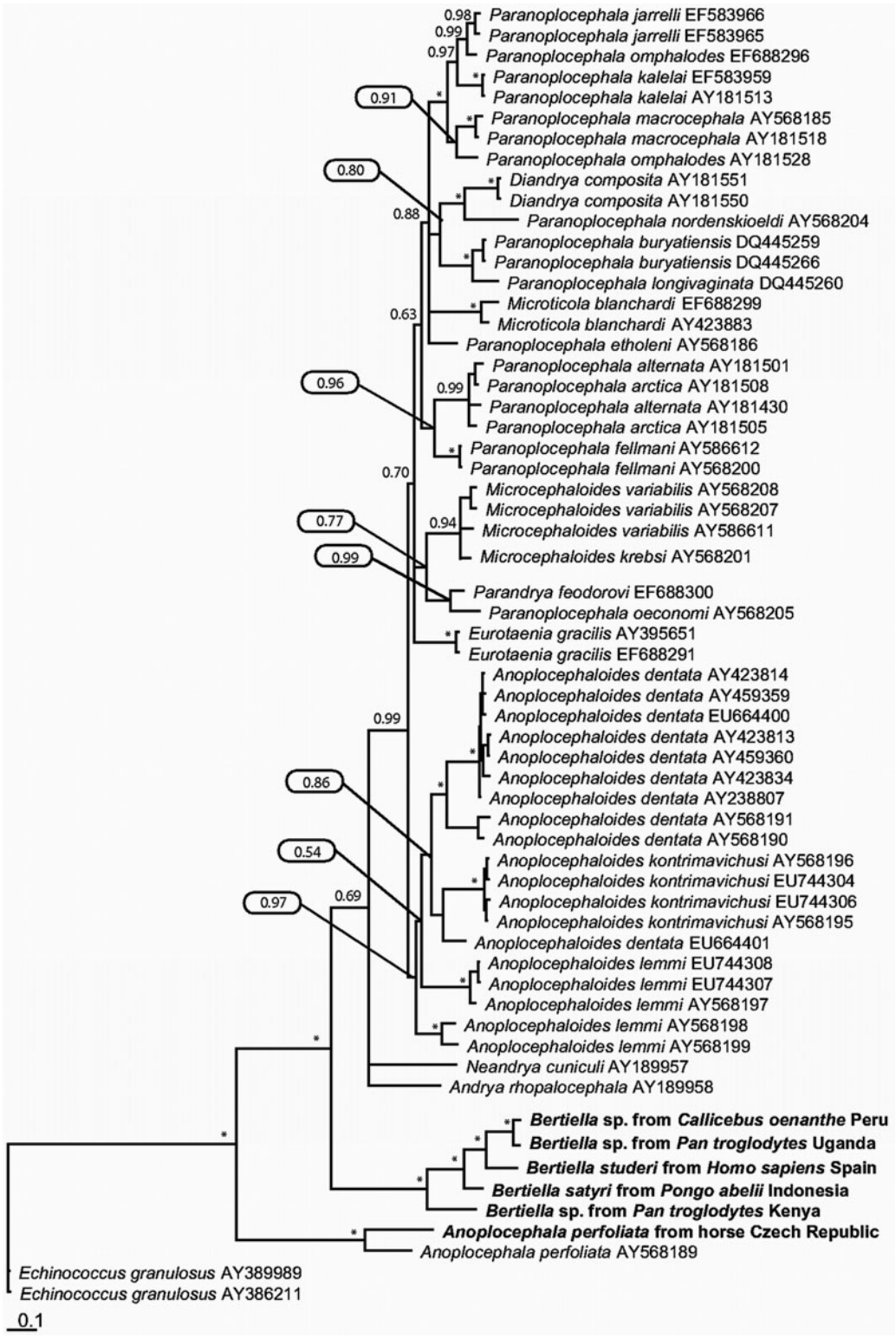

Fig. 2. Phylogenetic tree of 59 partial *cox*1 gene sequences of the subfamily Anoplocephalinae isolates. Sequences newly reported in the present study are in bold type and identified by name, host and locality of origin. Other sequences are identified by species name and GenBank accession number. The tree is rooted by *Echinococcus granulosus*. Posterior probabilities of BI are given above nodes; 1.00 support is indicated by an asterisk.

other genera of the subfamily, with *Bertiella* forming a sister lineage to the *Triplotaenia-Phascolotaenia-Progamotaenia* subclade (Fig. 1A). In contrast to this arrangement, the recent 28S rRNA analyses by Hardman *et al*. (2012) showed that the genus *Anoplocephala* forms a sister clade to the *Bertiella* isolates from marsupials and rodents (Fig. 1B). However, *Bertiella* species from primates, including the type species of the genus *B. studeri* originating from *P. troglodytes* (Blanchard, 1891), have never

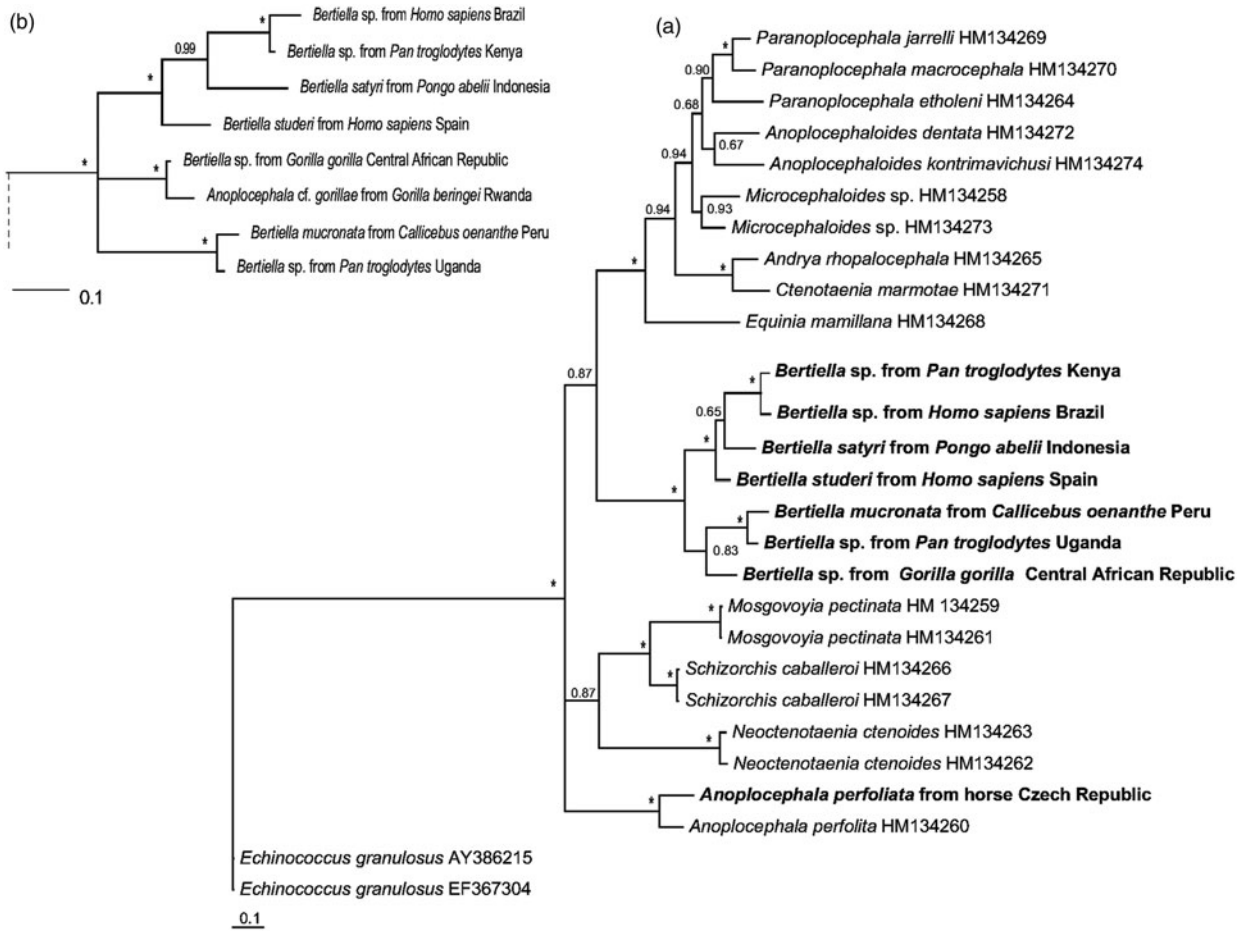

Fig. 3. Bayesian inference phylogenetic tree based on 25 partial *nad*1 gene sequences (747 bp) from various tapeworm isolates of the subfamily Anoplocephalinae (A). Sequences newly reported in the present study are in bold type and identified by name, host and locality of origin. Other sequences are identified by species name and GenBank accession number. The tree is rooted by *Echinococcus granulosus* (Taeniidae). Posterior probabilities of BI are given above nodes; 1.00 support is indicated by an asterisk. (A, B). Topology of the *Bertiella* isolates that include the isolate from mountain gorilla after the trimming of *nad*1 sequences to 366 bp prior Bayesian analysis (B).

been included in any previous analyses. Therefore, to amend the phylogenetic arrangement of the genus *Bertiella*; we added two new isolates from primates to the dataset of Hardman *et al*. (2012). These isolates clearly represented genus *Bertiella*, as confirmed by comparison of our sequence data with morphologically identified samples from previous studies (Galán-Puchades *et al*. 2000; Gómez-Puerta *et al*. 2009; Foitová *et al*. 2011). Surprisingly, position of these isolates (Fig. 5) confirmed neither the monophyly of *Bertiella*, nor its presumed close relationship to the genus *Anoplocephala*, to which *Bertiella* from marsupials and rodents remained as the sister group, as inferred by Hardman *et al*. (2012). Such discrepancy could be explained by existence of paralogous forms of DNA in *Bertiella* formed due to genome duplications, as proposed to have happened in other cestodes, e.g. *Ligula intestinalis* Bloch, 1782 and *Atractolytocestus huronensis* Anthony, 1958 (see Bouzid *et al*. 2008; Králová-Hromadová *et al*. 2010). On the other hand, there were none of the commonly encountered hints present in our sequence data, which are indicative of paralogs, such as double peaks in chromatograms or whole portions thereof unreadable due to confounded sequences (Griffin *et al*. 2011; El-Sherry *et al*. 2013). Thus, the paraphyly between *Bertiella* from primates and Australian marsupials and rodents can be regarded as a genuine phylogenetic relationship. Supportive of such a hypothesis can be a fact mentioned by Beveridge (1985, 1989) that almost all *Bertiella* species in marsupials and rodents differ morphologically from *Bertiella* in primates in mutual position of the uterus and osmoregulatory canals (medullary only in the latter group). This presumed difference between *Bertiella* species from primates and marsupials/rodents corroborated by our phylogenetic analysis, however, may not be so strict because this character was found also in the African species *Bertiella douceti* from West African scaly-tailed squirrels of the genus *Anomalurus* Waterhouse, 1843 (Beveridge 1985, 1989). The hypothesis of acquisition of this rodent parasite from primates sharing the same habitat (Baer, 1953), as

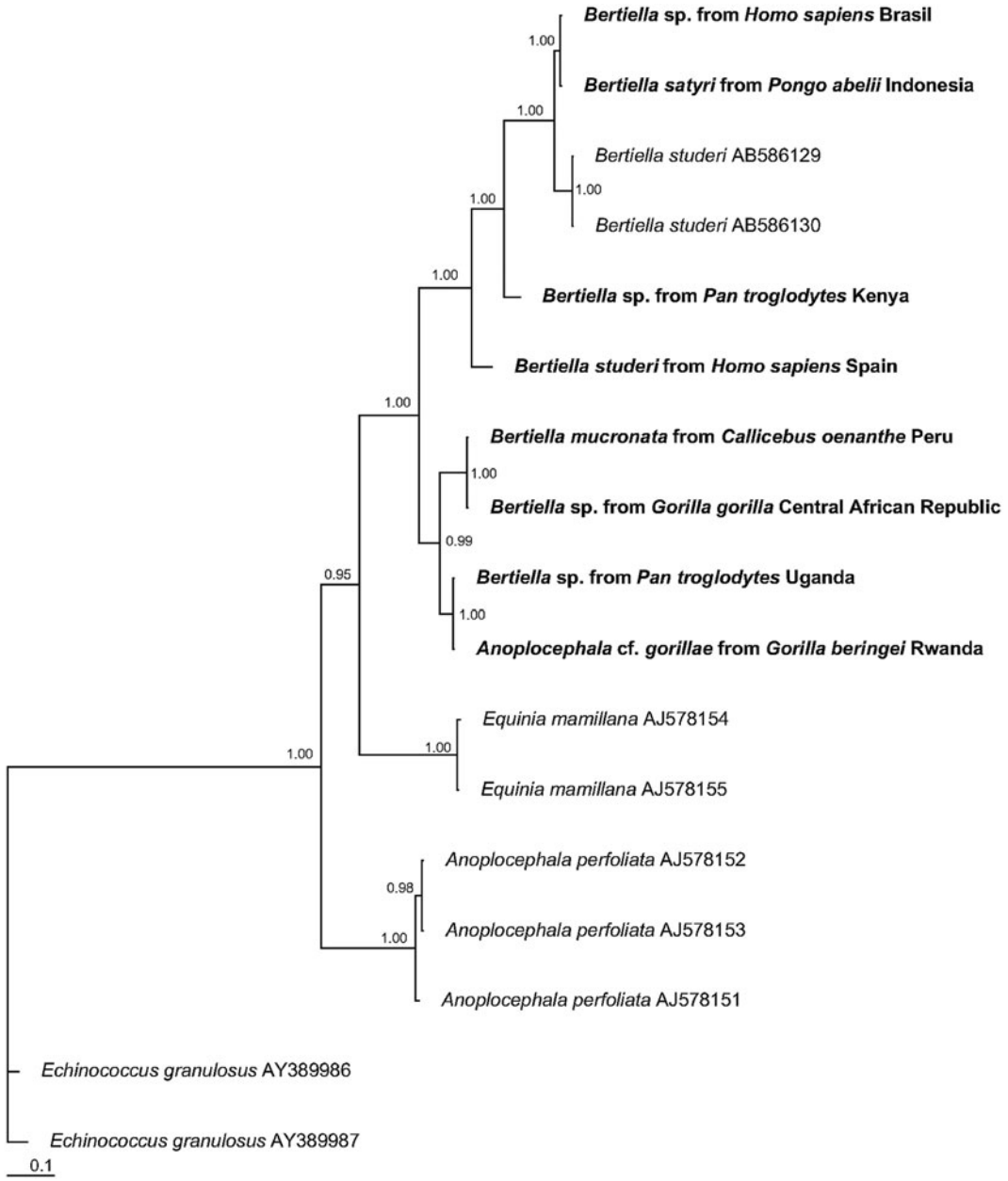

Fig. 4. Phylogenetic tree of 15 partial 5.8S-ITS2 rRNA gene sequences of isolates from man and primates and selected species of subfamily Anoplocephalinae. Sequences newly obtained in the present study are in bold type and identified by name, host and locality of origin. Other sequences are identified by species name and their GenBank accession number. The tree is rooted by Echinococcus granulosus. Posterior probabilities of BI are given above nodes.

well as any presumptions on co-phylogeny, calls for a deeper analysis.

Although the egg morphology (data not shown) of our isolate from *G. beringei* corresponds to *A. gorillae* described by Nybelin (1927) and Eilenberger (1998) and also is more similar to *A. perfoliata* from horses, in molecular phylogenies it invariably emerged within the *Bertiella* clade. This situation can be explained either as finding of *Bertiella* sp. newly in mountain gorillas, or, what is more probable, it is a discrepancy between phylogenic position of *A. gorillae* and its taxonomic placement. In latter case, a reclassification of *A. gorillae* to the genus *Bertiella* would solve the problem, however, additional molecular data resulting from well determined material are necessary for final solution.

Molecular markers are undoubtedly a powerful tool for distinguishing individual isolates, but the virtual absence of well-preserved adult tapeworms prevents unambiguous species identification. Although the analyses showed *Bertiella* spp. from primates monophyletic, the paraphyletic relationship of *Bertiella* isolates from primates and marsupials/rodents suggests that genus *Bertiella,* as traditionally acknowledged, and might actually be split into two genera. Then, species included in

Table 2. Pairwise genetic distance of *cox*1 sequences within (red colour) and among (green colour) chosen cestode species (whole analysis not shown). Newly obtained isolates from man, non-human primates and horse are bold typed

	PK1	PK2	MB1	MB2	EG1	EG2	MV1	MV2	MV3	AL	AD1	AD2	AD3	AK	**PE**	**UG**	**ES**	**ID**	**KE**	**CZ**
PK1																				
PK2	0·002																			
MB1	0·110	0·108																		
MB2	0·099	0·097	0·044																	
EG1	0·103	0·102	0·125	0·118																
EG2	0·103	0·102	0·118	0·118	0·011															
MV1	0·115	0·113	0·122	0·113	0·110	0·112														
MV2	0·118	0·117	0·123	0·113	0·112	0·113	0·020													
MV3	0·118	0·117	0·131	0·120	0·113	0·115	0·057	0·057												
AL	0·131	0·130	0·149	0·130	0·122	0·125	0·123	0·122	0·118											
AD1	0·112	0·113	0·135	0·122	0·122	0·122	0·122	0·125	0·118	0·128										
AD2	0·113	0·115	0·138	0·128	0·125	0·125	0·120	0·120	0·117	0·128	0·007									
AD3	0·115	0·117	0·135	0·122	0·122	0·122	0·118	0·118	0·112	0·123	0·007	0·010								
AK	0·123	0·122	0·136	0·120	0·099	0·102	0·117	0·118	0·123	0·113	0·112	0·115	0·113							
PE	0·169	0·167	0·176	0·172	0·163	0·163	0·176	0·179	0·177	0·163	0·167	0·169	0·167	0·172						
UG	0·167	0·166	0·174	0·171	0·161	0·161	0·174	0·177	0·176	0·161	0·166	0·167	0·166	0·171	0·002					
ES	0·164	0·163	0·172	0·167	0·149	0·149	0·166	0·166	0·167	0·156	0·161	0·163	0·161	0·164	0·023	0·021				
ID	0·179	0·177	0·197	0·199	0·174	0·172	0·186	0·184	0·190	0·181	0·186	0·187	0·182	0·181	0·077	0·076	0·076			
KE	0·161	0·159	0·172	0·171	0·161	0·163	0·154	0·151	0·159	0·159	0·164	0·163	0·164	0·166	0·105	0·103	0·107	0·117		
CZ	0·202	0·200	0·218	0·207	0·204	0·207	0·199	0·195	0·212	0·199	0·197	0·200	0·199	0·204	0·177	0·176	0·169	0·182	0·176	
AP	0·194	0·192	0·197	0·192	0·187	0·184	0·172	0·172	0·189	0·195	0·171	0·174	0·176	0·184	0·190	0·192	0·182	0·184	0·177	0·113

PK, *Paranoplocephala kalelai*; MB, *Microticola blanchardi*; EG, *Eurotaenia gracilis*; MV, *Microcephaloides variabilis*; AL, *Anoplocephaloides lemmi*; AD, *Anoplocephaloides dentate*; AK, *Anoplocephaloides kontrimavichusi*; AP, *Anoplocephala perfoliata*; ES, Spain; ID, Indonesia; PE, Peru; UG, Uganda; KE, Kenya; CZ, Czech Republic.

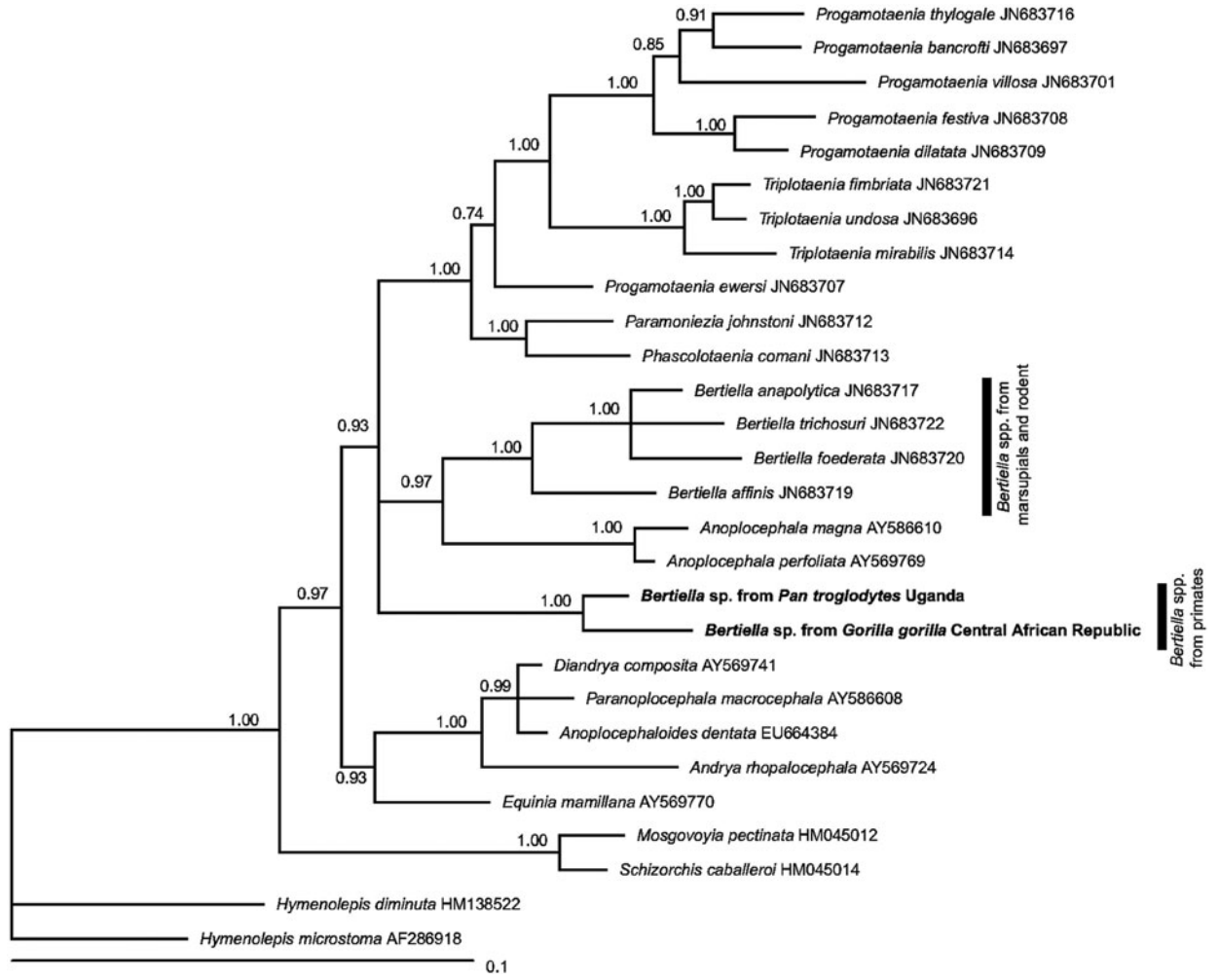

Fig. 5. Phylogenetic tree of 26 partial 28S gene sequences of the subfamily Anoplocephalinae isolates. Sequences newly reported in the present study are in bold type and identified by name, host and locality of origin. Other sequences are identified by species name and GenBank accession number. The tree is rooted by *Hymenolepis* spp. Posterior probabilities of BI are given above nodes.

these genera and host specificity of individual members need to be evaluated using combined morphological and molecular data. The available data indicate that man and other primates can be parasitized by several *Bertiella* species, though a clear species concept has yet to be elaborated based on good-quality material suitable for morphological study. We conclude that humans and other primates may share different *Bertiella* species at any place where local ecological conditions allow the transmission of these anoplocephalid cestodes.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S003118201500058.

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